Tokushiro TAKASO*: A developmental study of the integument in Gymnosperms (1) *Ginkgo biloba* L.

高相徳志郎*: 裸子植物の珠皮の発生学的研究 (1) イチョウ

Introduction Characters of integuments and seed coats have been studied not only for the classification of seed plants including fossils but also in considering their phyletic relationships. It is, however, very recent that in several taxa of Angiosperms detailed observations were made on histological features throughout the initiation of the integuments and subsequent development into the seed coats (Boesewinkel and Bouman, 1967; Bouman, 1971a, 1974, 1977; etc.). Bouman and his collaborators considered that developmental studies on the integuments would give much more persuasive evidences for the classification when compared with anatomical descriptions fragmentarily made so far. In the latest papers (Bouman, 1974, 1975; Bouman and Calis, 1977), furthermore, they referred to the evolutionary changes of the integuments based on the results of their studies.

Most of the histological studies of Bouman et al. mentioned above were restricted within Angiosperms. We have now little informations on the integuments of Gymnosperms.

I have been studying histological features of the integuments and the associated tissues or organs (e.g., nucellus, aril, collar, etc.) in some representative groups of Gymnosperms. This paper, which is concerned with the Ginkgoaceae comprising only one species, *Ginkgo biloba* L., is the first of a series in which the results of the studies will be presented. The Ginkgoaceae is considered to be one of unique taxa in Gymnosperms in having the dichotomous pattern in leaf venation, a peculiar morphology of the female reproductive organ, a spermatozoid and so forth (Hirase, 1896; Chamberlain, 1935; etc.). Concerning histological features of the integument of this family, however, only fragmentary and inadequate observations have been made (Strasburger, 1872, 1879; Quisumbing, 1925; Pankow and Sothmann,

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1967).

Materials and methods Materials were collected from certain three individuals which were planted in the campus of Chiba University, Chiba, and Komazawa Park, Tokyo. To obtain female reproductive organs at various stages of development, the serial collection was made between early March and middle April. Some of the materials were fixed with formalin-acetic alcohol (FAA), and the others with 2% glutaraldehyde solution.

Most of the materials fixed with FAA were replaced and stocked in 50% ethanol a few days later. For observation by light microscope, they were dehydrated through a tertiary-butyl alcohol series and embedded in paraplast. After serially sectioned 10 μm in thickness, they were stained with Heidenhain's haematoxylin, safranin and fast green. Those fixed with 2% glutaraldehyde solution as well as a part of the materials fixed with FAA were utilized for observation by scanning electron microscope (SEM). Before coated with gold and scanned, they were dehydrated in an ethyl alcohol series and dried in a critical point dryer with carbon dioxide. Both the materials killed by above two sorts of fixative gave proper figures for observation in a low magnification.

Observations and discussion A female reproductive organ, which was called an "ovuliferous structure" by Foster and Gifford (1959, 1974), arises either in the axil of a bract or in that of a foliage leaf. The ovuliferous structure usually consists of the following three parts: (1) an ovular part where two ovules are borne, (2) a part forming rim-like projection named "collar" below each ovule and (3) a stalk part supporting the former two.

The two ovules on the ovular part initiate on its abaxial side. At an early stage of development, these twin ovules look toward the abaxial side of the ovuliferous structure (Fig. 1A). During subsequent stages of development, however, they turn their faces in such directions as to become more apart from each other. Thus, at maturity the two ovules look toward the opposite directions each other (Fig. 1B).

Ovule primordium: Viewed from outside, the apex of the very young ovuliferous structure is slightly divided into two, each representing one ovule primordium. At this stage, the whole surface of the ovuliferous structure is smooth, i.e., without marked projections and depressions. At a little later stage, however, several distinct "ridges" appear at certain places

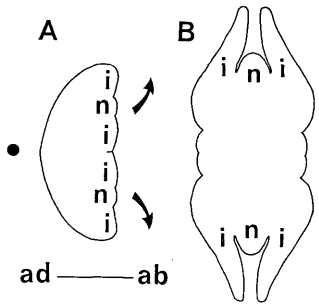

Fig. 1. Cross sections of twin ovules showing change of the direction toward which the ovules look. A. Two young ovules initiate with their apices looking toward the abaxial side of the ovuliferous structure. B. Two mature ovules look toward opposite sides each other. Arrows indicate the direction toward which the growing ovules turn their faces. (*ab*: abaxial side; *ad*: adaxial side; *i*: integument; *n*: nucellus and *c*: collar. These abbreviations are common in the following figures.)

on the adaxial side of the upper part of the ovuliferous structure (Fig. 7D; Strasburger, 1872). Additionally, a slight depression may frequently be observed on the abaxial side of the subapical part of the each ovule primordium (see an arrow in Fig. 7A). In radial longisections through the middle of the ovule primordium, the surface layer cells on the abaxial side of the subapical part are much varied in size and shape (see cells marked *a* in Fig. 2B). Beneath the surface layer lie many rows of cells which are vertical to the apical surface layer and considered resulting from repeated periclinal divisions of cells in the internal tissue (see cells marked *b* in Fig. 2B).

The histological features in the apical part of the ovule primordium differ from those of the vegetative shoot apex. Foster (1938) recognized a number of well-defined zones in the vegetative shoot apex. However, the zones comparable to those are not seen in the apical part of the ovule primordium. As described above, the ovule primordium has nothing but many vertical rows of cells in the internal tissue (Fig. 2B).

Initiation of integument: The first sign of the initiation of the integument is recognized as a "swelling" of tissue on the flank of the ovule primordium. At first, in each ovule primordium two swellings seem to be borne on the opposite side of the flank (see arrows in Fig. 7B) as described by Strasburger (1879). However, these two swellings are very small and the time when they are discernible is very short. The swelling soon extends around the nucellus resulting in a continuous circular swelling of tissue, that is, a young integument. By this stage of development, the two swellings which have firstly appeared become hardly distinguishable from

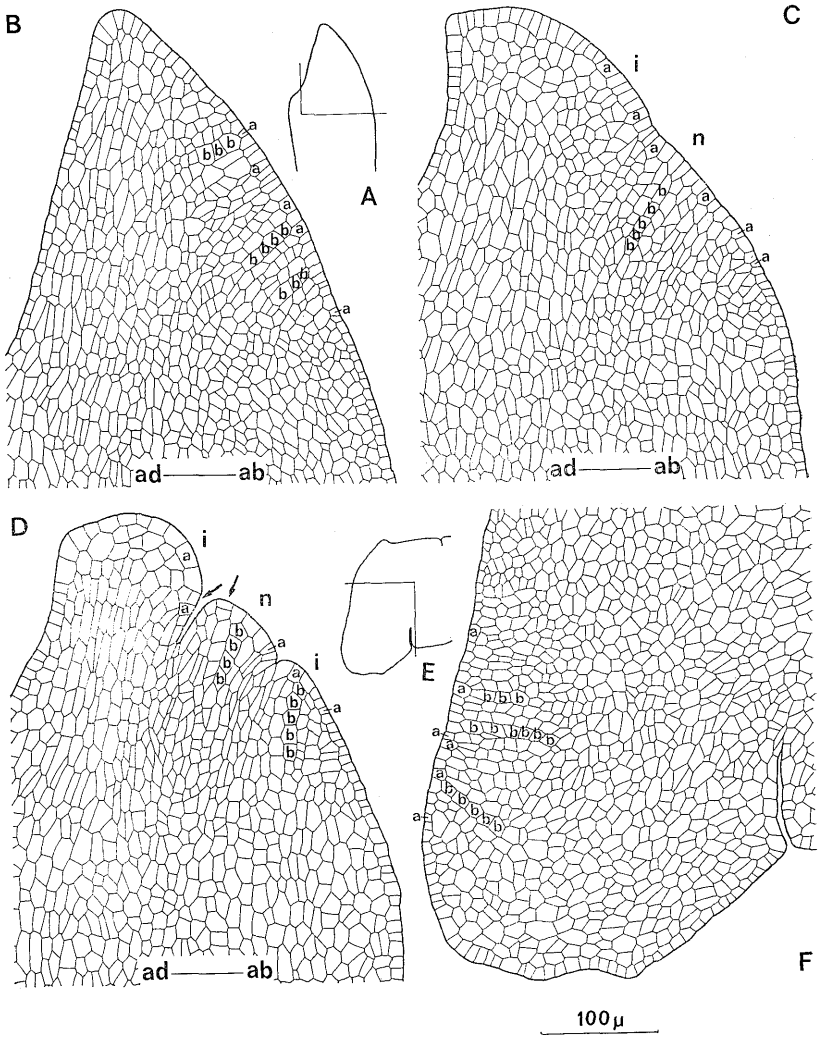

Fig. 2. Ovule primordia at various stages of development. A and B. A radial longisection through the middle of an ovule primordium before the initiation of the nucellus and integument. C. At the stage of the initiation of the integument. D. At the stage when the nucellus and integument are developing. Arrows point out periclinal cell divisions. E and F. A cross section of an ovule primordium at the stage of the initiation of the integument. Cells marked *a* are varied in size and shape, and cells marked *b* are arranged in rows.

surrounding tissues.

During the early stage of development the ovule primordium involves no changes in the histological features. The following characteristic features are observed at both stages, when the initiation of the integument is taking place (Fig. 2C, F) and when the young integument is growing (Fig. 2D). The apical surface layer of the ovule primordium is composed of various cells in size (marked *a* in the figure) and the internal tissue except for the boundary between the integument and the nucellus has many vertical rows of cells (marked *b*).

Development of integument: In young ovules, both the nucellus and integument grow rapidly. The growth of the integument is, however, more predominant than that of the nucellus (Figs. 2D and 3). Thus, the whole

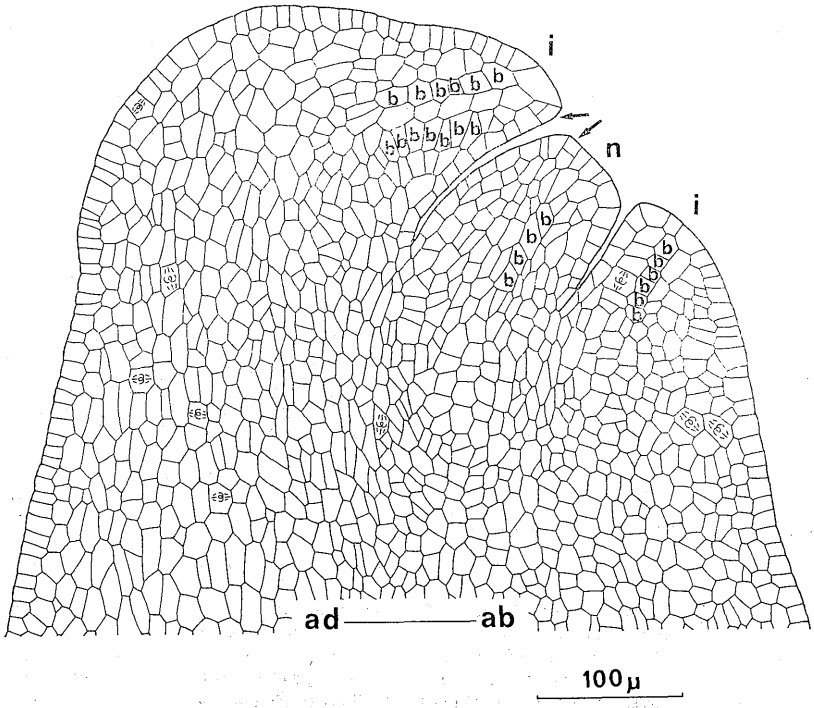

Fig. 3. Radial longisection through the middle of a young ovule. Arrows point out periclinal cell divisions. Cells marked *b* are arranged in rows.

of the nucellus comes to be enveloped by the integument (Fig. 7C). A growing integument is tapering with cells at its tip being much larger and frequently accumulating tannin (Fig. 5B).

Most of the surface layer cells of the growing ovule divide anticlinally, and yet some, particularly at the apical parts of the integument and nucellus, may divide periclinally (see cells pointed out by arrows in Fig. 3, for example). Cells in the internal tissue undergo exclusively periclinal divisions as in the stage of the initiation of the integument, forming many rows of cells (see cells marked *b* in Figs. 2D and 3). Presence of the many rows of cells in the internal tissue was already stated by Quisumbing (1925), and this histological feature is most remarkable in the integumentary tissue. Some of internal cells lying near the base of the integument may divide in other direction, i.e. anticlinally, resulting in an increase in thickness of the basal part of the integument (see dividing cells in Fig. 3).

Initiation of collar: Initiation of the collar can be recognized in the same way as that of the integument, that is, the collar also appears as a swelling of tissue though it arises just below the base of the integument (see an arrow in Fig. 4B; Pankow and Sothmann, 1967). The surface layer cells of the swelling usually divide anticlinally, scarcely do periclinally (Table 1) as recognized in median longisections through the growing ovule (Fig. 4B, D). Cells in the internal tissue, however, may often divide periclinally as reported by Pankow and Sothmann (1967), and this manner of cell division is responsible for the swelling of tissue.

As stated already, some ridges develop on the adaxial side of the upper part of the ovuliferous structure (Fig. 7D). In the young ovuliferous structure the ridges resemble the early swellings of the collar in both the external and internal morphology, so that it is very difficult to distinguish the collar from the ridges at the early stage of development. Owing to this circumstance, it is uncertain where the first sign of the initiation of the collar appears.

Regarding the order of the initiation of the integument and collar, Pankow and Sothmann (1967) reported that the integument appeared earlier than the collar. However, this is not true on every side of the ovuliferous structure and should be limited only to the abaxial side. On the adaxial side the order can not be ascertained because the early swellings of the

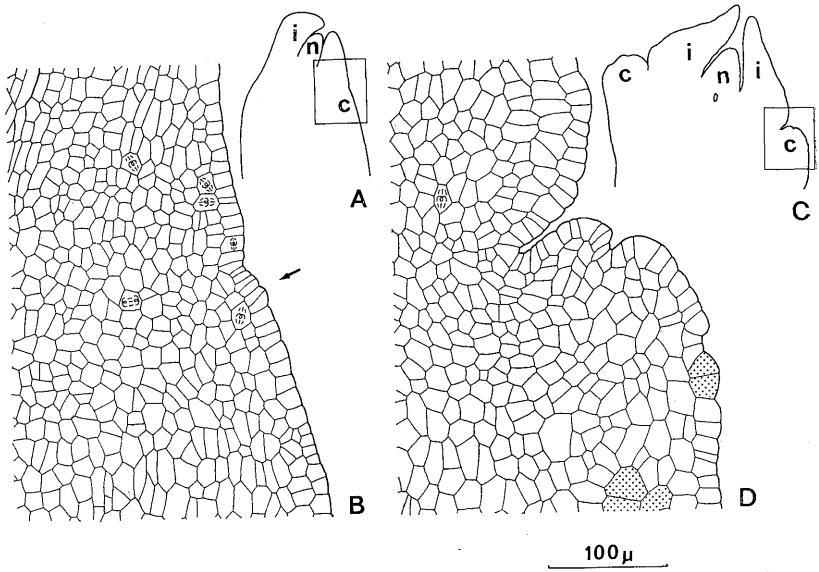

Fig. 4. Development of the collar. A and B. A radial longisection through the middle of a growing ovule, showing the detail of the collar part in B. C and D. A radial longisection through the middle of a mature ovule, showing the collar part in detail (D). Dotted cells indicate being well stained with dyes.

collar are not distinguishable from the ridges as stated above.

Development of collar: The growth of the collar proceeds largely by means of periclinal and anticlinal divisions of internal cells, but simultaneously it accompanies successive anticlinal divisions of the surface layer cells to some extent (Figs. 4D and 7E; Pankow and Sothmann, 1967). Through the development of the collar, periclinal divisions of the surface layer cells rarely occur (Table 1).

A developed collar usually has two or three bulges at its apical part (Fig. 4C, D). Pankow and Sothmann (1967) interpreted that the bulges were already formed at the stage of the initiation of the collar; yet in the present observations the collar has no bulges when it is initiating (Fig. 4B). I thus consider that the bulges are secondarily formed either by the division of the tip of the collar during its development, or by the growth of some of the ridges on the ovuliferous structure.

Most of cells constituting the ridges on the ovuliferous structure appear

Table 1. The frequency of periclinally divided cells seen in the respective apical surface layers of the nucellus, integument and collar throughout their initiation and subsequent development. The number of cells examined for calculation of the frequency is bracketed. See text for explanation.

	Stage I	Stage II	Stage III
Nucellus	1.2% (255)	1.8% (850)	1.4% (560)
Integument	0.2% (510)	1.1% (830)	2.0% (440)
Collar	——	0.1% (850)	0.1% (1080)

to participate in the formation of the collar, and the rest in the formation of a part of the integument. Thus, the ridges diminish with the development of the ovuliferous structure finally to be indistinguishable. The ridges shown in Fig. 7F, which have been still remained on some places of the collar, also disappear during the subsequent development of the collar.

Mature ovuliferous structure: In the mature ovuliferous structure, the integument envelops the whole of the nucellus and forms a micropyle (Fig. 5). Though the shape of the micropyle is circular or oval (Strasburger, 1872; Haan, 1920; etc.), the apical part of the integument is somewhat lobed as shown in Fig. 7G. The base of the integument is much thicker than the base of the nucellus (Fig. 5A). The collar becomes a stout structure with a thick base and slightly envelops the base of the integument (Figs. 5A, B and 7G). Internal cells of the mature collar become somewhat enlarged (Fig. 5B).

Crater-shaped protuberance on the ovuliferous structure: As pointed out by an arrow in Fig. 7H, a small protuberance is often observed in a relatively large depression on the adaxial or lateral side of the upper part of the growing ovuliferous structure. Of protuberances the larger ones usually may have an additional small depression in its center, so it looks crater-shaped. In mature ovuliferous structure, more developed protuberances are often seen (Fig. 7I). The organographic significance for these protuberances is not clear.

Frequency of periclinally divided cells in the respective surface layers of

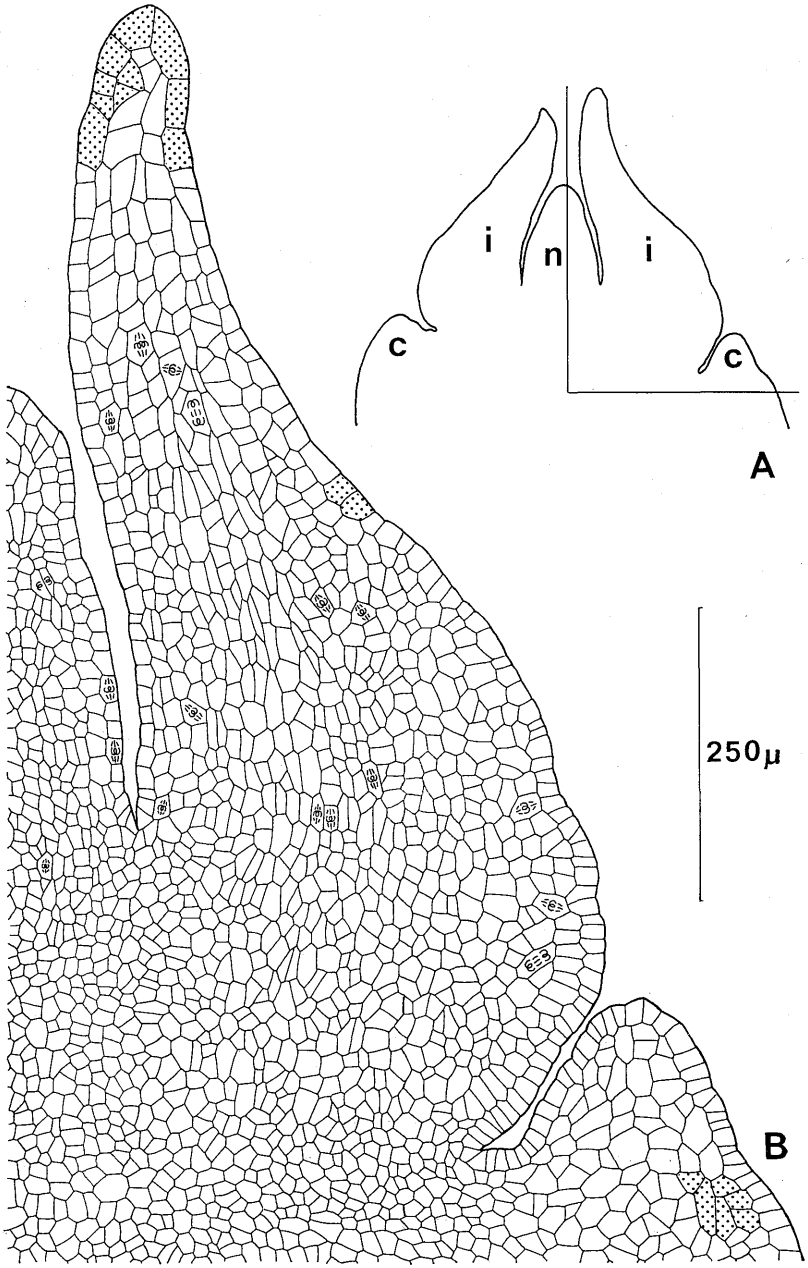

Fig. 5. Radial longisection through the middle of a mature ovule and collar. Dotted cells indicate being well stained with dyes.

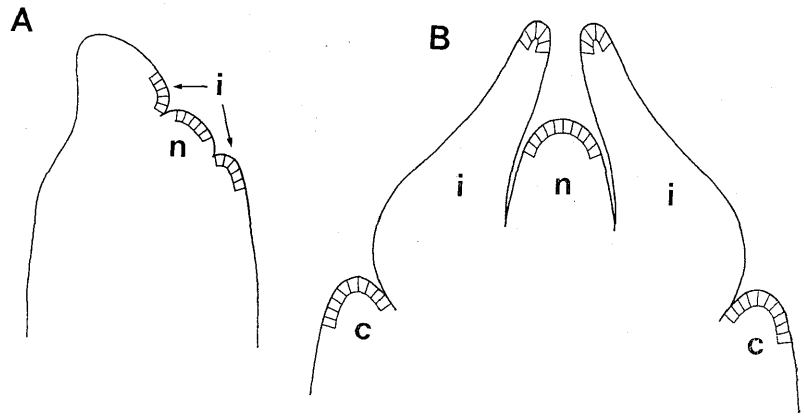

Fig. 6. Radial longisections through the middle of ovules illustrating portions where cells are examined for calculating the frequency of periclinal divisions. A. A young ovule. B. A mature ovule. See text for explanation.

the nucellus, integument and collar: As stated above, the surface layer cells of the young ovuliferous structure usually repeat anticlinal divisions, but the cells of the respective apical surface layers of the nucellus, integument and collar often divide periclinally. In order to show details on this respect, frequency of periclinally divided cells in these portions is examined and summarized in Table 1.

For the purpose of comparison of the frequency among different stages of development as well as among different portions, the whole of developmental stages of the ovuliferous structure is conveniently divided into three. Stage I is from the stage when the ovule is primordial (Fig. 2B) to the stage of early development of the ovule (Fig. 2D); Stage II, followed from Stage I to about the stage when the collar initiates (Fig. 4A); Stage III, followed from Stage II to the mature stage of the ovuliferous structure (Fig. 5). Periclinally divided cells in the surface layers are counted in three sections which are taken at intervals of a section from five serial median longisections through the ovule primordium or the growing ovule. The number of cells examined in one section is slightly different, depending upon positions and developmental stages as shown in Fig. 6: in the nucellus, 5 cells per section were ovserved in Stage I, and 10 cells per section in

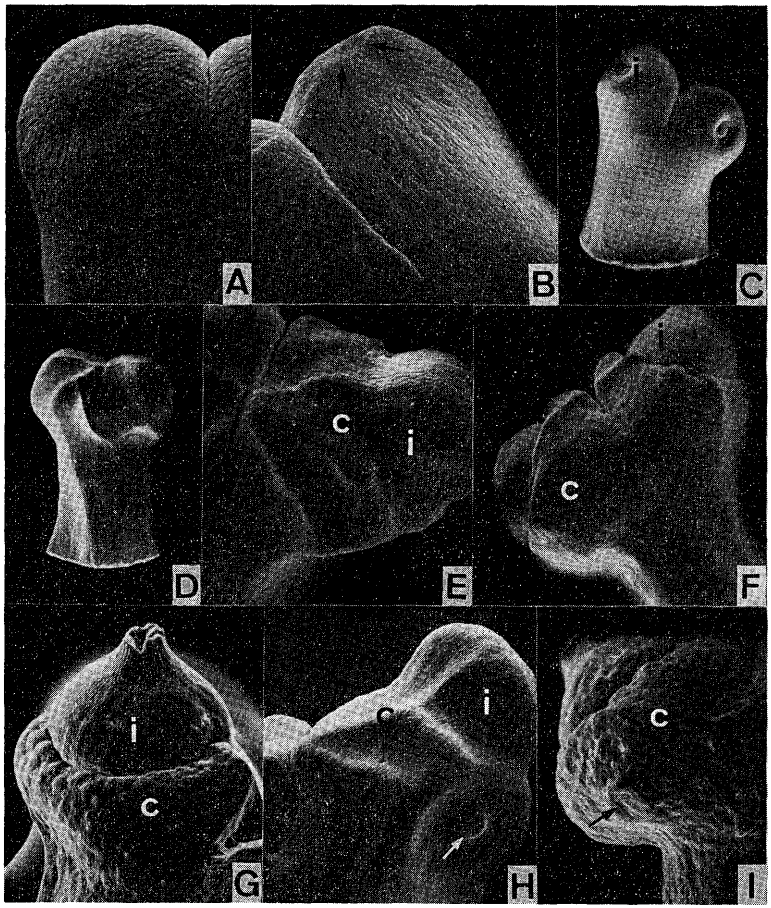

Fig. 7. External appearance of parts of the ovuliferous structures at various stages of development (A-C and G : abaxial view ; D-F, H and I : adaxial view). A. At the stage with a young ovule primordium. An arrow points out a slight depression. ×65. B. A little later stage of A. The integument is initiating (arrows). ×55. C. At the stage with growing ovules. ×30. D. Ridges on the ovuliferous structure. ×30. E. The collar part in center. ×30. F. At the stage with highly developed ovules. ×20. G. At the stage with a mature ovule and collar. ×20. H and I. The protuberances shown in some examples (arrows). ×30, 20.

both of Stages II and III; in the integument, 10 cells per section were observed in all of Stages I, II and III not only because in one section the integument is seen on both sides of the nucellus, but also because in later

stages only several cells in the apical surface layer of the integument divide periclinally. Similarly, in the collar 20 cells per section were examined in both of Stages II and III.

As seen in the table, the frequency of periclinally divided cells in the surface layer of the nucellus is almost constant throughout all of the stages of development. However, slightly higher frequency (1.8%) was noted in Stage II than in other stages of development; thus, Stage II is considered to represent the most conspicuous growing period of the nucellus. In the integument, the frequency becomes higher as the development proceeds: 0.2% in Stage I, 1.1% in Stage II and 2.0% in Stage III. Practically, however, in Stage II and especially in Stage III, cells just dividing are very rare in the apical surface layer. From this fact it seems that the surface layer of the integument at these later Stages II and III dose not show higher activity in cell division. In the collar, on the other hand, much lower frequencies (0.1%) are obtained in both of Stages II and III.

In comparison of the nucellus, integument and collar, there is a discernible difference among them in the frequency of periclinally divided cells in their respective surface layers. Higher frequency is recognized in the nucellus than in the collar throughout all the stages of development. The frequency in the integument is intermediate between the two.

In these three different portions of the ovuliferous structure, daughter cells which result from periclinal divisions of cells constituting the apical surface layers also divide periclinally, and in the resultant daughter cells moreover the same manner of cell division is undergone in succession. Thus, if the origin of internal tissues is traced back to the ovule primordium, some amount of internal cells in both the nucellus and integument is found to have been derived from the apical surface layers.

Acknowledgements I wish to express my thanks to Dr. M. Ono of Tokyo Metropolitan University and to Dr. M. Nishida of Chiba University for their suggestions and guidance. Thanks are also due to Dr. H. Tobe of Chiba University for his valuable discussions and criticisms.

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珠皮及び種皮は種子植物の分類及び系統的類縁関係を考察する上で重要な形質とされてきたが，これまで珠皮の組織発生に関する研究は被子植物の幾つかの分類群に限られており，裸子植物についてはほとんどなされていない。こうした理由から，著者は裸子植物の幾つかの代表的分類群において珠皮の組織発生学的研究を続けており，その結果を順次報告するつもりである。

第1報のイチョウでは ovuliferous structure（以下 O. S.）が通常2つの胚珠（それぞれに1枚の珠皮が発達する）とその各々の下部に発達する collar 及びこれらを支えている長い柄から構成されている。胚珠と collar の initiation と発達様式について次のような観察結果が得られた。2つの胚珠は O. S. の上部の背軸側から共に軸の反対方向を向いて initiation をするが，その後の生長の間に胚珠が互いに逆方向に反転するため，成熟した2つの胚珠は全く反対の向きに並ぶ。珠皮と collar の initia-

tion は共に若い O.S. に組織の“もり上がり”として認められ，initiation の順序は O.S. の背軸側で珠皮が collar より早いが，向軸側では確かめられなかった。珠皮の initiation は異なる2か所からごく小さなもり上がりとして同時に起こるように見えるが，initiation 後すぐに連続したリング状のもり上がりとなって珠心の基部をとり囲む。胚珠原基及び若い胚珠では，表層の細胞に大きさと形においてかなり変異が見られ，また表層より内側の組織には表層に対して垂直な多数の細胞列群が見られる。この細胞列群は表層より内側の細胞の並層分裂の繰り返しによってできると思われる。珠皮の伸長は表層より内側の細胞の並層分裂の繰り返しによって起こり，厚さは垂層分裂の繰り返しによって増す。表層の細胞には O.S. の生長期間を通じて垂層分裂が見られるが，珠心，珠皮及び collar の先端の表層には並層分裂も観察される。この並層分裂の頻度は生長期に無関係に珠心で最も高く，珠皮，collar の順に低くなる。成熟した O.S. では，珠皮は珠心を完全に包んで珠孔を形成し，collar は珠皮の基部をわずかに包む。クレータ状の形をした隆起がしばしば O.S. の向軸側あるいは側部に見られる。

○コガシアズマザサの新変種（鈴木貞雄） Sadao Suzuki: A new variety of *Sasaella kogasensis* (Nakai) Nakai ex Koidzumi

東京の園芸家の間でコクマザサと称して鉢植えにした高さ 10 cm ほどの小さなササが出廻っている。それは牧野富太郎博士のコクマザサ *Sasa albo-marginata* (Makino) Makino et Shibata f. *minor* Makino〔植物学雑誌 15: 25 (1901)〕でないことは葉がずっと狭く，披針形で軟らかく，かつ下面に軟毛が密生していることですぐにわかる。肩毛は基部だけが粗渋で，ほかは全く平滑であることからアズマザサ属 *Sasaella* のものである。私は露地植えにして数年間栽培してみた。稈は高さが 30～40 cm，直径 1～2 mm となり，1節から1枝を分岐する。その後は毎年新条がでてもそれ以上には大きくならない。稈鞘は開出する長毛と逆向する細毛が密に混生し，節間は逆向の細毛があり，しばしば無毛，葉鞘は開出する長毛と細毛が密生し，しばしば細毛だけが密生する。葉は披針形または線状披針形，長さ 9～12 cm，幅 13～17 mm，紙状膜質，下面に軟毛が密生し，上面に長毛が散生するか，または無毛である。このような形質は関東地方北部から東北地方南部にかけてよく見られるコガシアズマザサ *Sasaella kogasensis* (Nakai) Nakai ex Koidzumi にきわめてよく似ているが，それは稈が高さ 1～2 m，直径が 4～8 mm に達してやや剛壮である。

いわゆるコクマザサはその後，鉢植えにされるほか東京都をはじめその周辺の神奈川県や千葉県の諸所で庭木の根じめや石付など，園芸的によく利用されていることがわかった。コガシアズマザサによく似ているが，それよりはるかに小形で繊細である